REMARKS

This is meant to be a complete response to the Office Action mailed September 16, 2010. In the Office Action, the Examiner rejected claims 31-37, 42, 45, 46, 48-51, 60 and 61 under 35 U.S.C. 112, first paragraph (enablement), and rejected claim 42 under 35 U.S.C. 112, second paragraph. Also in the Office Action, the Examiner issued the following rejection under 35 U.S.C. 103(a): (1) claims 31-37, 42, 45, 46, 49-51, 60 and 61 over US 5,482,841 in view of McClusky et al. (J. Immunol. (1988) 141:1451-1455), Prilliman et al. (Immunogenetics, 1997, 45:379-385), DiBrino et al. (Biochemistry (1995) 34:10130-10138), Hausmann et al. (Clin. Exp. Immunol. (1993) 9:183-188) and Chen et al. (J. Immunol. (1994) 152:2874-2881); and (2) claim 48 over the '841 patent in view of McClusky et al., Prilliman et al., DiBrino et al., Hausmann et al., and Chen et al., and further in view of Nakajima and Yaoita (Nucleic Acids Res. (1997) 25:2231-2232).

Applicants' Response to the 35 U.S.C. 112 Rejections

In the Office Action, the Examiner rejected claims 31-37, 42, 45, 46, 48-51, 60 and 61 under 35 U.S.C. 112, first paragraph (enablement), and rejected claim 42 under 35 U.S.C. 112, second paragraph.

Claim 42 has been canceled herein, and therefore the rejections thereof have been rendered moot.

In response to the 35 U.S.C. 112, first paragraph rejection, claims 31, 35-36, 48-50 and 61 have been amended herein.

Thus, Applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. 112, first and second paragraph rejections of the claims as now pending.

Applicants' Response to the First 35 U.S.C. 103(a) Rejection

In the Office Action, the Examiner rejected Applicants' claims 31-37, 42, 45, 46, 49-51, 60 and 61 under 35 U.S.C. 103(a) as being unpatentable over US 5,482,841 in view of McClusky et al. (J. Immunol. (1988) 141:1451-1455), Prilliman et al. (Immunogenetics, 1997, 45:379-385), DiBrino et al. (Biochemistry (1995) 34:10130-

10138), Hausmann et al. (Clin. Exp. Immunol. (1993) 9:183-188) and Chen et al. (J. Immunol. (1994) 152:2874-2881). Applicants respectfully traverse the rejection based on the amendments to the claims and for the reasons stated herein below.

The presently disclosed and claimed inventive concept(s) is directed to a method for detecting the presence of anti-class I MHC complexes. In the method, a pool of functionally active, recombinantly produced, truncated individual soluble class I MHC trimolecular complexes that have been purified substantially away from other proteins is obtained. ***Each complex present in the pool contains the same truncated, individual class I MHC heavy chain molecule.*** At least one soluble class I MHC trimolecular complex from the pool is then linked to a substrate (directly or indirectly) such that the trimolecular complex retains the physical, functional and antigenic integrity of a native complex. A sample is then reacted with the substrate/complex, and the substrate is washed to remove unbound portions of the sample. The substrate/complex is then reacted with means for detecting anti-class I MHC antibodies, and it is determined that anti-class I MHC antibodies specific for the individual class I MHC molecule are present in the sample if the means for detecting the anti-class I MHC antibodies is positive.

The pool of functionally active, recombinantly produced, truncated individual soluble class I MHC trimolecular complexes is obtained by isolating mRNA encoding a class I MHC heavy chain allele from a source, reverse transcribing the mRNA to obtain cDNA, and identifying an individual class I MHC heavy chain allele in the cDNA. The identified allele is then subjected to PCR amplification. The PCR amplification results in a PCR product that encodes a soluble form of the desired class I MHC heavy chain molecule, whereby the PCR product does not encode the cytoplasmic and transmembrane domains of said molecule. The PCR product is then inserted into a mammalian expression vector that is inserted into a mammalian cell line. The mammalian cell line is then cultured under conditions which allow for expression of the recombinant individual soluble class I MHC heavy chain molecule, endogenous loading of peptide ligand and noncovalent association of beta-2-microglobulin, thus producing soluble class I MHC trimolecular complexes. Said complexes are then purified

substantially away from other proteins while maintaining the physical, functional and antigenic integrity of the native MHC trimolecular complex.

US 5,482,841 is the only reference provided by the Examiner that is directed to methods for detecting anti-MHC antibodies; the '841 patent discloses an assay to determine the presence of antibodies or other receptors specific for alloantigens. The '841 patent discloses extracting HLA from a cellular source with a mild detergent and partially purifying by precipitation of potentially interfering components, and then using such extracted HLA in a sandwich assay to determine the presence of receptors specific for the HLA extracted from the cells. However, the HLA extracted by the methods of the '841 patent are ***a mixture of HLA molecules***, and such mixture is neither characterized nor separated, so that the identity and specificity of the HLA molecules are not determined. Therefore, the '841 patent does not teach, disclose or suggest the claim limitation of **"each complex present in the pool containing the *same* truncated, individual class I MHC heavy chain molecule".**

In addition, the detergent solubilization methods utilized in the '841 patent yield low amounts of HLA, and the lysis of the entire cell to obtain HLA ***introduces all cellular proteins, as well as lipids and other cell components, which means additional protein purification and loss of HLA***.

As background to the disadvantages and defects of the prior art, multiple methods of creating class I MHC molecules were known prior to the present invention; however, said methods suffered from several defects and disadvantages, as discussed in greater detail herein below. In the subject application, Applicants describe and claim a method of detecting the presence of anti-class I MHC antibodies that utilizes soluble class I MHC trimolecular complexes that are produced by a method that is completely different from other known methods. The main difference between prior art production methods and the class I MHC complex production method utilized in the presently claimed inventive concept(s) is that the soluble class I MHC trimolecular complexes produced in the presently claimed method are naturally assembled within the cell to form heterotrimers comprising the recombinantly introduced heavy chain,

naturally or endogenously produced light chain (β2m), and naturally produced and endogenously loaded antigenic peptides, and are purified substantially away from other proteins. Once properly assembled by the host cell, the soluble class I MHC trimolecular complexes produced in accordance with the method of the present inventive concept(s) is secreted outside of the cell, thus enabling their purification away from other proteins.

One of the advantages of the presently claimed inventive concept(s) over the prior art is the fact that the MHC trimolecular complexes are recombinantly truncated so that the complexes are no longer membrane bound but rather are soluble and thus secreted from the cell, thereby greatly aiding in the ability to purify the individual, soluble MHC trimolecular complexes away from other proteins in sufficient amounts as well as retain the activity (i.e., the conformation) thereof without denaturing the complexes. Prior art references such as US 5,482,841, DiBrino et al., Hausmann et al. and Chen et al. utilize detergent solubilization, which has a variety of disadvantages over the presently claimed inventive concept(s). The most important disadvantage is that MHC/HLA complexes purified by detergent solubilization will include a mixture of HLA molecules and thus do not provide a method for purifying **individual**, soluble MHC trimolecular complexes as recited by the present claims. This disadvantage is especially true for DiBrino et al., where the cell line in which HLA-B*4403 was produced also expresses HLA-Cw4 as well as HLA-B35 (see Zemmour et al.). Therefore, the methods of DiBrino et al. cannot possibly produce an **individual**, soluble MHC trimolecular complex, as the methods of DiBrino et al. (i.e., W6/32 immunoaffinity chromatography) do not distinguish between the three different HLA molecules produced by the methods taught therein.

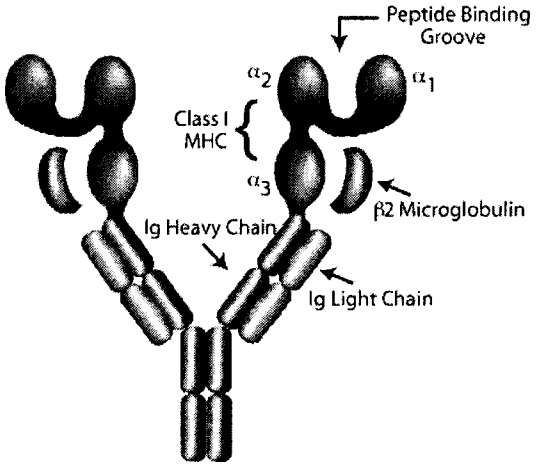
In addition, another advantage of the presently disclosed and claimed inventive concept(s) over these prior art references that teach detergent solubilization is that the prior art methods of detergent solubilization result in lysis or **killing of the cells** in order to obtain the MHC molecule. In contrast, the mammalian cell line producing secreted MHC complexes in accordance with the present inventive concept(s**) remain alive and therefore continue to produce class I MHC complexes**.

As the Examiner is surely aware, the prior art is good for everything it teaches, not just the invention it describes or claims. EWP Corp. v. Reliance Universal, Inc., 225 U.S.P.Q. 20, 25 (Fed. Cir. 1985) ("on the issue of obviousness, the combined teachings of the prior art as a whole must be considered.") Furthermore, it is impermissible within the framework of Section 103 to pick and choose from any reference only so much of it as will support a given position, to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one of ordinary skill in the art. Bausch & Lomb, Inc. v. Barnes-Hind/Hydrocurve, Inc., 230 U.S.P.Q. 416,420 (Fed. Cir. 1990). As such, the entirety of the teachings of the '841 patent and the DiBrino et al., Hausmann et al. and Chen et al. references may not be ignored or "excluded" from the other parts of said references. Therefore, Applicants respectfully submit that when the teachings of the '841 patent and the DiBrino et al., Hausmann et al. and Chen et al. references are considered as a whole, the requirement that the cells utilized therein be detergent solubilized (and thus lysed or killed) actually teaches away from the claimed recited step of "harvesting the soluble class I MHC complexes from the culture while retaining the mammalian cell line in culture for production of additional soluble class I MHC complexes". Thus, the '841 patent and the DiBrino et al., Hausmann et al. and Chen et al. references cannot be combined with any other references to render the claimed inventive concept(s) obvious.

The Examiner has recognized the deficiencies of the primary reference (i.e., the '841 patent) as well as the secondary references of DiBrino et al., Hausmann et al. and Chen et al., and has attempted to supply same with the teachings of McCluskey et al. and Prilliman et al. McCluskey et al. teach the formation of a hybrid molecule that fuses the N-terminal domains of one protein to the C-terminal domains of a secreted protein. For example, this approach has been utilized by fusing functional domains of one protein to structural domains of the secreted immunoglobulin molecule. In this way, one can utilize the secreted properties of the immunoglobulin molecule (or any other naturally secreted protein) and the functional properties of another protein. There is no teaching for how a secreted protein is to be created. Rather, the goal is to fuse the

intrinsic secreted properties of one protein to the functional domain of a protein that is typically not secreted.

Any number of secreted proteins could be fused to the functional domains of an MHC molecule. For example, the senior author of the McCluskey et al. reference is David Margulies. Dr. Margulies played a key role in the dimerX techolology, whereby the HLA class I molecule is expressed as a soluble protein through fusion to an Ig molecule. For example, the recombinant HLA-A2:Ig fusion protein maintains the MHC N-terminal functional domains fused to an Ig in order to get secreted.



In the cited reference, McCluskey et al. fuse the alpha-1 and alpha-2 domains of a classical (H-2K, D, or L) class I MHC molecule to the C-terminal domains of a secreted non-classical MHC molecule. McCluskey et al. teach the formation of a **hybrid** molecule. Secretion of an MHC molecule is obtained by creating this hybrid molecule. McCluskey et al. do not teach the use of mutagenesis to create a secreted version of the native molecule. Rather, McCluskey et al. teach the fusion of portions of a native cell surface MHC molecule to the C-terminal portion of a secreted non-classical molecule.

It is important to note that McCluskey et al. do not explore the nature of the truncation site – they do not test to see if secretion of an MHC protein can be obtained without using the nonclassical Q10 molecule. Moreover, the Q10 molecule used includes not only a gene sequence that encodes the extracellular protein sequence, but

said sequence **also includes exons and introns for the transmembrane domain (TM) protein sequence as well as exons/introns encoding intracytoplasmic domains** of the Q10 gene. Therefore, this secreted hybrid molecule is much larger than the soluble class I MHC trimolecular complex produced according to the claimed method, and the effects of the addition of the Q10 molecule has not been evaluated. That is, it has not been shown that the hybrid molecule has the same structural characteristics of the native class I MHC, it has not been shown that the hybrid molecule binds beta-2-microglobulin and epitope normally, and it has not been shown that the hybrid molecule does not possess different and unusual functions when compared to the native class I MHC. McCluskey et al. teach the fusion of a secreted protein to a native MHC molecule, and thus do not teach the mutagenesis of an HLA molecule so that it is secreted in its native form with no transmembrane region, as required by the presently claimed invention.

As stated herein above, the prior art is good for everything it teaches, and it is impermissible within the framework of Section 103 to pick and choose from any reference only so much of it as will support a given position, to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one of ordinary skill in the art. As such, the teachings of the use of a full-length class I MHC molecule (i.e., containing transmembrane and cytoplasmic domains) may not be ignored or "excluded" from the other parts of the McCluskey et al. reference, and the effect of these domains on the hybrid molecule has not been established by McCluskey et al. Therefore, Applicants respectfully submit that the McCluskey et al. reference clearly teaches away from the claimed method, in which the cytoplasmic and transmembrane domains of the class I MHC heavy chain molecule have been removed.

Prilliman et al. is the closest prior art to Applicants' inventive concept(s), as it is Applicants' earlier publication related to preliminary work done by the Applicants prior to the presently disclosed and claimed inventive concept(s). However, this preliminary work was performed to isolate the ***peptides*** presented in class I MHC heavy chain

molecules, and does not provide a method of producing soluble individual class I MHC trimolecular complexes.

In making an obviousness determination, there are three factual inquiries to be analyzed: (1) the scope and content of the prior art are to be determined; (2) differences between the prior art and the claims at issue are to be ascertained; and (3) the level of ordinary skill in the pertinent art resolved. *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966). The Federal Circuit developed this test to guard against impermissible hindsight reconstruction:

> [t]o imbue one of ordinary skill in the art with knowledge of the invention ... when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher.
> *W.L. Gore & Associates v. Garlock, Inc.*, 721 F.2d 1540, 1553 (Fed. Cir. 1983).

> A factfinder should be aware, of course, of the distortion caused by hindsight bias and must be cautious of arguments reliant upon *ex post* reasoning.
> *KSR Intl'l Co. v. Teleflex, Inc.*, 127 S. Ct. 1727, 1742 (2007)

> Hindsight is not a justifiable basis on which to find that ultimate achievement of ***a long sought and difficult scientific goal*** was obvious.
> *Amgen, Inc. v. Chugai Pharmaceutical Co., Ltd.*, 927 F.2d 1200, 1209, 18 USPQ2d 1016, 1023 (Fed. Cir. 1992) (emphasis added)

The inventive concept is part of the "unpredictable art" of biotechnology, and the fact that the Examiner has combined a total of SIX references (some of which teach away from the claimed limitations), all of which published more than four years prior to the priority claim of the subject application, clearly demonstrates that the Examiner is using hindsight reconstruction as the basis for her obviousness determination. Therefore, Applicants respectfully submit that the Examiner has not established a *prima facie* case of obviousness. Applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. 103(a) rejection of currently pending claims 31-37, 45-46,

49-51 and 60-61 as being unpatentable over the above-stated combination of references.

Applicants' Response to the Second 35 U.S.C. 103(a) Rejection

In the Office Action, claim 48 was rejected under 35 U.S.C. 103(a) as being unpatentable over the '841 patent in view of McClusky et al., Prilliman et al., DiBrino et al., Hausmann et al., and Chen et al., and further in view of Nakajima and Yaoita (Nucleic Acids Res. (1997) 25:2231-2232).

Applicants respectfully submit that independent claim 31, from which claim 48 depends, is non-obvious over the combination of US 5,482,841 in view of McCluskey et al., Prilliman et al., DiBrino et al., Hausmann et al. and Chen et al., for the reasons discussed herein above in response to the first 35 U.S.C. 103(a) rejection. As Nakajima and Yaoita do nothing to supply the deficiencies of the rejection of the independent claim, Applicants respectfully submit that dependent claim 48 is also non-obvious over the combination of US 5,482,841 in view of McCluskey et al., Prilliman et al., DiBrino et al., Hausmann et al., Chen et al., and Nakajima and Yaoita. Applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. 103(a) rejection of claim 48 over said combination of references.

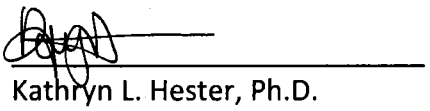
CONCLUSION

This is meant to be a complete response to the Office Action mailed September 16, 2010. Applicants respectfully submit that each and every rejection of the claims has been overcome. Further, Applicants respectfully submit that claims 31-37, 45-46, 48-51 and 60-61, as now pending, are patentable over the art of record and are in a condition for allowance. Favorable action is respectfully solicited.

Further, upon allowance of any of claims 31-37, 45-46, 48-51 and 60-61, Applicants respectfully request that all withdrawn species (including currently withdrawn claims 38-41) be rejoined and reconsidered by the Examiner.

Should the Examiner have any questions regarding this Amendment, or the Remarks contained therein, Applicants' representative would welcome the opportunity to discuss same with the Examiner.

Respectfully submitted,



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